

# Sex attractant for the banana moth, *Opogona sacchari* Bojer (Lepidoptera: Tineidae): provisional identification and field evaluation

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## Abstract

**BACKGROUND:** The banana moth, *Opogona sacchari* Bojer, is a polyphagous agricultural pest in many tropical areas of the world. The identification of an attractant for male *O. sacchari* could offer new methods for detection, study and control.

**RESULTS:** A compound extracted from female *O. sacchari* elicited responses from antennae of male moths. This compound was identified as a 2/3,(Z)13-octadecadienal by gas chromatography-mass spectrometry. An analog, 2/3,(Z)13-octadecadienol, was also detected in some extracts at roughly a 1 : 20 ratio (alcohol : aldehyde) but did not elicit responses from antennae of male moths. Electroantennograms of synthetic candidate dienals found the strongest responses from (Z,Z)-2,13-octadecadienal and (E,Z)-2,13-octadecadienal. In field trials, (E,Z)-2,13-octadecadienal attracted more male *O. sacchari* than (Z,Z)-2,13-octadecadienal. Attraction was not improved for either of these compounds when the corresponding stereoisomeric alcohol was added at ratios of 1 : 1, 1 : 10 or 1 : 100 (alcohol : aldehyde). Jackson sticky traps containing 250 µg lures of (E,Z)-2,13-octadecadienal caught as many males as did traps holding virgin females.

**CONCLUSION:** (E,Z)-2,13-octadecadienal has been identified as an attractant for *O. sacchari* males and can be used as a monitoring lure of populations of this moth.

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**Keywords:** *Opogona sacchari*; banana moth; attractant; octadecadienal

## 1 INTRODUCTION

The banana moth, *Opogona sacchari* Bojer (Lepidoptera: Tineidae), is an agricultural pest in many tropical areas of the world.<sup>1,2</sup> Affected crops include banana, coffee, pineapple, papaya, bamboo, maize, sugarcane, stored tubers and ornamental crops, including *Dracaena*, *Cordyline*, *Chamaedorea* palms, *Philodendron* and *Cycas revolute*.<sup>2</sup> Larvae usually feed internally, burrowing within the stem or roots of host plants. This species was originally described from the Mascarene Islands in the Indian Ocean in 1856. However, during the last 30 years, it has been reported from humid tropical and subtropical areas in Africa, Europe and South America.<sup>3</sup> In the United States, *O. sacchari* is recorded only from Florida and Hawaii, and has been in Hawaii since 1990 or before.<sup>3</sup>

*Opogona sacchari* is a generalist feeder, and infestations typically begin on diseased, damaged or dead tissue, or on other organic material associated with the crop or commodity. However, older larvae can burrow into healthy plant tissues, such as stems of *Dracaena* sp. (Hollingsworth RG, personal observation). There is evidence that *O. sacchari* is expanding its host range in Hawaii. During the past 8 years, it has been noted as a root/stem feeder on orchids and coffee seedlings being grown in potting media high in organic matter (Hollingsworth RG, personal observation). Extensive damage to pineapple plantings has also been recorded recently,<sup>4</sup> with such severe damage to some varieties that they are now avoided by the pineapple industry in spite of their

otherwise desirable horticultural traits. In addition, *O. sacchari* is responsible for serious damage to coffee trees, caused by stem girdling following pruning activities.<sup>5</sup>

In the United States, *O. sacchari* is federally regulated as a quarantine pest.<sup>6</sup> Therefore, *O. sacchari* can be a direct pest and an indirect pest on horticultural commodities exported from Hawaii, because of the risk of commodity rejection if larvae are found by quarantine inspectors. Commodities exported from Hawaii to the US mainland that are sometimes found to be infested with *O. sacchari* include palms and *Dracaena* (Hollingsworth RG, unpublished data), banana,<sup>7</sup> rambutan, mangoes and sweet potatoes (Follett P, private communication, 2009). Current techniques for monitoring banana moth populations require sampling for larvae through destructive assessment of the crop. Identification of a sex pheromone attractant for *O. sacchari* would provide a non-destructive population-monitoring tool. This attractant could be used to study the seasonality and distribution of banana moths in various host crops, and to facilitate timing

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of pesticide applications with peak pest abundance. Additionally, pheromone-based tools such as pheromone disruption could be used as part of a control program for this pest.

Early work to investigate a possible pheromone for *O. sacchari* was undertaken by Rotundo and Tremblay<sup>8</sup> and Ionedá *et al.*<sup>9,10</sup> Both groups found that male moths were behaviorally responsive to extracts from calling females. However, neither group identified the active pheromone components. Rotundo and Tremblay<sup>8</sup> used electroantennography (EAG) to assay several compounds known to be pheromone components of other Lepidoptera, and found that (Z)-11-hexadecenal (Z11-16:Ald) elicited responses from moth antennae, but field work described in their paper was never published. Field attraction of *Opogona* spp. to a 99 : 1 mixture of (Z)-8-dodecenyl/(E)-8-dodecenyl acetates was, however, noted during a study in Malawi with the tortricid *Cryptophlebia batrachopa* (Meyrick).<sup>11</sup> In Florida, traps baited with virgin females were used to monitor populations of *O. sacchari* in greenhouses over a 1 year period.<sup>2</sup> These results clearly indicate the existence of a female-produced sex pheromone in *O. sacchari*.

Although *Opogona* sp. sex pheromones have not been identified, pheromones for other tineid moths have been identified and utilized for purposes of control.<sup>12–19</sup> The majority of the identified pheromones are 2/3,(Z)13-octadecadienyl compounds, predominantly alcohols, aldehydes and acetates. Although the moths attracted to these pheromones belong to different subfamilies within Tineidae, it is reasonable to postulate that the *O. sacchari* pheromone is similar in structure. The objective of the present study was to characterize, synthesize and field test sex pheromone attractants for *O. sacchari*.

## 2 MATERIALS AND METHODS

### 2.1 Insects

Insects used in tests were taken from a colony of *O. sacchari* that had been in continuous laboratory culture since 1999, originally collected from infested rambutan bark in Kurtistown, Hawaii. The colony is maintained within ventilated plastic containers (mixed-age colony) provisioned with artificial diet on an as-needed basis (continuous production cycle). The artificial bean- and wheat-based diet (modified from Follett and Lower<sup>20</sup>) contained the following proportions of blended ingredients: 468 g cooked great northern beans, 120 g wheat meal, 70 g yeast flakes, 7.0 g ascorbic acid, 4.4 g sodium benzoate, 2.2 g sorbic acid, 40 g agar and 1400 mL hot water.

### 2.2 Gland extractions

Female moths, 2–3 days old, were frozen at  $-70^{\circ}\text{C}$  while exhibiting calling behavior 2–4 h prior to the onset of photophase. Abdominal tips were subsequently excised and extracted for 5 min in hexane. Hexane extracts were then transferred into conical glass vials and evaporatively concentrated under a purified nitrogen stream.

### 2.3 Gas chromatography–mass spectrometry

Extracts from calling female moths were analyzed by gas chromatography–mass spectrometry (GC-MS) using two instruments. The first, located in Hawaii, consisted of an Agilent Technologies 6890N gas chromatograph interfaced to a Hewlett-Packard 5973 mass selective detector equipped with either an HP-5 MS or a DB-255 MS column (both  $30\text{ m} \times 0.25\text{ mm ID}$ ,  $0.25\text{ }\mu\text{m}$  film thickness). The standard temperature program used was 80 to  $240^{\circ}\text{C}$

at  $10^{\circ}\text{C min}^{-1}$ , with a 1 min start delay with the injector temperature set at  $250^{\circ}\text{C}$ , using helium as a carrier gas ( $1.1\text{ mL min}^{-1}$ ). The second instrument, located in Virginia, was a Hewlett Packard G1800A GCD system equipped with an HP-5 MS column ( $30\text{ m} \times 0.25\text{ mm ID}$ ,  $0.25\text{ }\mu\text{m}$  film thickness). The temperature program used was 60 to  $250^{\circ}\text{C}$  at  $10^{\circ}\text{C min}^{-1}$ , with a 1 min start delay with the injector temperature set at  $250^{\circ}\text{C}$ , using helium as a carrier gas ( $1.0\text{ mL min}^{-1}$ ).

### 2.4 Electroantennography

Electroantennographic responses were recorded either with a gas chromatograph–electroantennogram coupled system (GC-EAD) or with an electroantennogram (EAG) set-up only. Male electroantennographic responses to female pheromone gland extracts were recorded using an Agilent Technologies 6890 gas chromatograph coupled to a Syntech electroantennogram detector system (Hilversum, The Netherlands). The GC was equipped with an HP-5 column ( $30\text{ m} \times 0.25\text{ mm ID}$ ,  $0.25\text{ }\mu\text{m}$  film thickness) with helium as carrier gas ( $2.3\text{ mL min}^{-1}$ ) and make-up gas ( $10\text{ mL min}^{-1}$ ), which were combined with a Y-type connector. A Graphpack-3D/2 flow splitter (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) was attached to the base of the connector, and the effluent was split 1 : 1 between the flame ionization detector (FID) and the electroantennogram detector (EAD) via a heated transfer line ( $250^{\circ}\text{C}$ ). The injector, in splitless mode, and FID were held at 250 and  $275^{\circ}\text{C}$  respectively. The oven temperature program began at  $80^{\circ}\text{C}$  for 1 min, and was then ramped at  $10^{\circ}\text{C min}^{-1}$  to  $240^{\circ}\text{C}$  and was held for 13 min. Whole moth heads or excised antennae were secured with electrode gel (Spectra 360; Parker Laboratories, Inc., Fairfield, NJ) between the parallel metal paddle electrodes of a Syntech EAG probe antenna holder (5 mm separation between paddles). Humidified air was passed over the antennal preparation and acted as a carrier for effluent from the EAD transfer line. The antennal signal generated by the EAD was amplified and filtered by a Syntech NL 1200 high-impedance amplifier and analyzed with the FID signal using Syntech GC-EAD2000 software. Z11-16:Ald, previously shown to elicit EAG responses from *O. sacchari*,<sup>8</sup> was used to test the system.

Electroantennograms of single synthetic aldehyde compounds were recorded using the recording system described above. However, these recordings were taken with compounds directly introduced into the humidified airstream leading to the antennal preparation. This was accomplished by puffing air through a glass Pasteur pipet containing the compound of interest on filter paper. All replicates consisted of sequential puffs in the following order: air only ( $3\times$ , negative control), 1-hexanol (1-6:OH,  $3\times$ , positive control), three sequential puffs of each of the test aldehydes, air only ( $3\times$ ) and 1-6:OH ( $3\times$ ). The order of the aldehydes was randomized in each replicate. Aldehyde (100 ng) was dosed on the filter paper and allowed 30 s for the dichloromethane solvent to evaporate before puff dispensing. Only replicates that showed responses to 1-6:OH were included in the analysis.

### 2.5 Nuclear magnetic resonance

$^1\text{H}$  NMR spectra were obtained with a Bruker DRX-400 FT-NMR spectrometer equipped with a broadband gradient probe. All spectra were recorded in deuterated chloroform with 1% tetramethylsilane (TMS) as an internal standard.

### 2.6 Chemicals

All solvents and reagent compounds were purchased from Sigma-Aldrich, St Louis, Missouri or Acros Organics, Geel, Belgium. All

solvents were HPLC grade. (*E,Z*)-3,13-Octadecadienol (*E3*,Z13-18:OH) and (*Z,Z*)-3,13-octadecadienol (*Z3*,Z13-18:OH) were gifts from Scentry Biologicals, Inc., Billings, Montana. Both compounds were >95% pure by GC-FID analysis. (*E,Z*)-2,13-Octadecadienol (*E2*,Z13-18:OH) and (*Z,Z*)-2,13-octadecadienol (*Z2*,Z13-18:OH) were purchased from Pherobank, Wageningen, the Netherlands. Both compounds were >98% pure by GC-FID analysis. (*E,Z*)-2,13-Octadecadienol used in field test 3 was obtained from Pherobank, Wageningen, the Netherlands, >90% pure by GC-FID analysis.

## 2.7 Synthesis

Candidate 2/3,Z13-dienyl compounds known to have pheromonal activity in Tineidae were synthesized to facilitate further electrophysiological and field studies. (*Z,Z*)-2,13-Octadecadienal (*Z2*,Z13-18:Ald) was prepared from *Z2*,Z13-18:OH by Dess–Martin oxidation. *Z2*,Z13-18:OH (166 mg, 0.62 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was added to a solution of Dess–Martin periodinane (419 mg, 1.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (4 mL) and stirred at room temperature for 3 h.<sup>21</sup> The mixture was then diluted with diethyl ether (20 mL) and treated with saturated  $\text{NaHCO}_3$  solution (15 mL). The organic layer was removed, washed with water, dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The resulting oil was purified by column chromatography on silica gel with a gradient of hexanes: $\text{CH}_2\text{Cl}_2$  (100% hexanes to 1:1 to 100%  $\text{CH}_2\text{Cl}_2$ ) to yield *Z2*,Z13-18:Ald (118 mg, 72%). (*E,Z*)-2,13-Octadecadienal (*E2*,Z13-18:Ald), (*Z,Z*)-3,13-octadecadienal (*Z3*,Z13-18:Ald) and (*E,Z*)-3,13-octadecadienal (*E3*,Z13-18:Ald) were prepared from their corresponding alcohols by oxidation with pyridinium chlorochromate (PCC). A solution of one of the three octadecadienols (100 mg, 0.38 mmol) and PCC (200 mg, 0.93 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was stirred at room temperature for 4 h. The crude mixture was then purified by column chromatography on silica gel with a gradient of hexanes: $\text{CH}_2\text{Cl}_2$  (100% hexanes to 1:1 to 100%  $\text{CH}_2\text{Cl}_2$ ) to provide the desired aldehyde (71–79% yield).  $^1\text{H}$  NMR analyses of *Z2*,*E3*-18:Ald showed trace contamination (4%) of the corresponding *E2* aldehyde, indicating a slight isomerization of the *Z2* double bond. Isomerization was not detected with *E2*,Z13-18:Ald, *E3*,Z13-18:Ald or *Z3*,Z13-18:Ald by  $^1\text{H}$  NMR analysis.  $^1\text{H}$  NMR aldehyde spectra matched those previously reported.<sup>22</sup> GC-FID analysis showed less than 5% contaminants for each aldehyde (*Z2*,*E3*-18:Ald, *E3*,Z13-18:Ald and *Z3*,Z13-18:Ald isomerizations to *E2* were 88, 16 and 19% respectively). (*Z,Z*)-2,13-Octadecadienyl acetate (*Z2*,Z13-18:OAc), (*E,Z*)-2,13-octadecadienyl acetate (*E2*,Z13-18:OAc), (*Z,Z*)-3,13-octadecadienyl acetate (*Z3*,Z13-18:OAc) and (*E,Z*)-3,13-octadecadienyl acetate (*E3*,Z13-18:OAc) were prepared from their corresponding alcohols by esterification with acetic anhydride. A solution of one of the three octadecadienols (100 mg, 0.38 mmol), acetic anhydride (200  $\mu\text{L}$ , 2.1 mmol) and pyridine (1 mL) was stirred at room temperature overnight. The mixture was poured into cold 1 M hydrochloric acid (5 mL) and extracted with hexane (3  $\times$  5 mL). The organic extract was washed with saturated  $\text{NaHCO}_3$  (5 mL), dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. The resulting oil was purified by column chromatography to yield the desired acetate (67–73% yield). Acetate structures were confirmed by GC-MS analysis and comparison with previously reported fragmentation patterns.<sup>23</sup> All acetates were >98% pure by GC-FID analysis.

## 2.8 Field trials

Single candidate attractants and several binary mixtures were field tested in an abandoned papaya plantation near Keaau,

Hawaii (Hawaii Island), in 2007 and 2008. Surviving papaya trees were sparse, and the ground was covered with low-growing weeds. Banana moths in this field were reproducing on dead and dying papaya trunks. Jackson sticky cardboard traps (Better World Manufacturing, Fresno, CA) were used to trap male moths. Test lure compounds were applied in  $\text{CH}_2\text{Cl}_2$  to red rubber septa (5 mm; Kimble/Kontes, Vineland, NJ), and the septa were placed on the sticky inserts of Jackson traps. In traps used as positive controls, a virgin female (2–3 days old) was confined within a custom-made, aluminum window-screen enclosure (3 cm diameter  $\times$  12 cm long) attached to the ceiling of the trap along its length. Water was supplied to females by wetting dental wicks that had been glued to the inside of the enclosure. Traps were hung from trees  $\sim 1.5$  m above the ground in a double-row pattern, with each row holding a complete set of randomized treatments. Spacing within rows was 15–20 m, and rows were separated by 30 m. Males of *O. sacchari* were counted every 3–4 days. Sticky inserts and virgin females were replaced at that time. For each trapping experiment, four replications were carried out over time, with replications ranging from 7 to 18 days in duration (2–5 trapping periods each). New traps, new septa and a new randomization scheme were used in each replication. Field test 1 measured the attraction of males to chemical lures when compounds were used singly. Twelve different compounds were tested, comprising the *E2*,Z13-, *E3*,Z13,Z2,Z13 and *Z3*,Z13-octadecadienyl aldehydes, acetates and alcohols. Because of technical difficulties, *E3*,Z13-18:OH and *Z3*,Z13-18:OH were tested in only one of the four experimental replications. Therefore, these compounds were excluded from analyses, but results are reported. Field test 2 measured response of males to the two best-performing lures (*E2*,Z13-18:Ald and *Z2*,Z13-18:Ald) when these were used singly or blended with their corresponding alcohol at an aldehyde/alcohol ratio of 1:1, 1:10 or 1:100. All septa using single attractants held 250  $\mu\text{g}$  of the specified chemical. Septa holding blends contained 250  $\mu\text{g}$  of *E2*,Z13-18:Ald or *Z2*,Z13-18:Ald plus an additional amount (250, 25 or 2.5  $\mu\text{g}$ ) of its corresponding alcohol.

In a second papaya field under commercial production located less than 1 km away, a mass trapping trial (field test 3) was carried out in 2009 using red rubber septa baited with *E2*,Z13-18:Ald. Papaya trees in this field were about 2–3 m in height, with rows 3.15 m apart. Trees had begun bearing fruit <6 months prior to tests, and, because of the rocky soil, some trees fell over and rotted, providing breeding material for the banana moths collected in the traps. Efficacy of mass trapping was assessed by moth capture in Jackson traps holding virgin females, set up and monitored as previously described, except all Jackson traps were attached  $\sim 1$  m above the ground on bamboo stakes. A 4  $\times$  4 grid of traps (6.3 m spacing) holding virgin females was positioned at the center of a larger 5  $\times$  5 grid of traps (also spaced 6.3 m apart) baited with lures holding 50 mg each of *E2*,Z13-18:Ald. Thus each trap holding a virgin female was surrounded on four sides by artificial lure traps located  $\sim 4.5$  m away and baited with the artificial attractant. While virgin females were replaced every 3–4 days, septa were dosed at the beginning of the trial and were not replaced over the 2 week collection period. As an experimental control, an additional grid of 4  $\times$  4 traps holding virgin females was set up in the other half of the papaya field  $\sim 40$  m distant from the closest traps comprising the first grid.

## 2.9 Analysis

EAG results were analyzed using ANOVA followed by comparison of means using the Student–Newman–Keuls test. All analyses



of significance were made at the  $P < 0.05$  level. Field data on male trap captures were normalized by dividing trap captures by the number of days in the trapping period. The number of males caught per day in each trap, averaged over all trapping periods comprising each replication, was used as the dependent variable in ANOVA models, while replication, trapping row and treatment (lure type) were used as independent variables. Tukey's HSD test ( $\alpha = 0.05$ ) was used to compare means.<sup>24</sup> Using the same ANOVA model, field test 2 data were also analyzed after lure types had been recategorized as either those containing the *E2*/3,*Z*13 geometric isomer or those containing the *Z2*/3,*Z*13 isomer (with experimental controls deleted from the dataset). Casual observations suggested that a particular trap catching a high number of moths over one sampling period tended to catch high numbers of moths over subsequent sampling periods, relative to moth capture in nearby traps baited with the same attractant. To test this hypothesis, the authors calculated the difference in trap catch between pairs of traps baited with the same attractant for each trapping period (3–4 days duration) using data from field test 1 associated with the three artificial attractants catching the most moths. Within each trapping replication (comprising 2–5 trapping periods), the trap associated with the highest moth catch was designated as 'A', and the other trap was designated as 'B'; the difference 'A – B' in trap catch was then computed for all trapping periods, except for the trapping period associated with the highest trap catch. The 95% confidence interval for the mean difference was used as the significance test: if the interval did not include zero, this was considered as evidence that the specific location of an individual trap was an important variable affecting trap catch.

### 3 RESULTS

Coupled gas chromatography–electroantennogram detection (GC-EAD) analysis of female *O. sacchari* abdominal tip extracts revealed a single compound that elicited responses from antennae of male moths. GC-MS analysis of these extracts showed that the EAG active compound had a mass ion of 264 *m/z* and a fragmentation pattern consistent with a 2/3,*Z*13-octadecadienal (2/3,*Z*13-18:Ald).<sup>25</sup> An EAG inactive compound with a mass ion of 266 *m/z* and a fragmentation pattern consistent with a 2/3,*Z*13-octadecadienol (2/3,*Z*13-18:OH)<sup>23,25</sup> was also intermittently detected in a range near the detection limits of the instrument and was not definitively identified. The two compounds were detected roughly in a 1:20 ratio (alcohol:aldehyde). While the retention time of the 2/3,*Z*13-18:Ald matched that of *E2*, *Z*13-18:Ald on both a non-polar HP-5 MS and a polar DB-255 MS column, precise identification became difficult because many of these aldehydes are unstable and isomerize during GC analysis (even with cool on-column injection).<sup>22</sup>

Given the isomerization problems of 2/3,*Z*13-18:Ald, electroantennograms were recorded with synthetic aldehydes without GC separation. The results of this testing are displayed in Table 1. When grouped, the 2,13-dienals elicited stronger responses than the 3,13-dienals. There was not a significant difference in response between *Z2*, *Z*13-18:Ald and *E2*, *Z*13-18:Ald.

**Field test 1 (compounds tested singly).** The average catch of males per trap per day (MTD) in glue traps across treatments ranged from 1.82 to 0.008 (Table 2). ANOVA results indicated significant differences in male capture associated with replications ( $P < 0.03$ ,  $F = 3.1$ ,  $df = 3, 80$ ) and treatments (lure types) ( $P < 0.0001$ ,  $F = 21$ ,  $df = 11, 80$ ) but not trapping row ( $P = 0.8$ ,  $F = 0.08$ ,  $df = 1, 80$ ).

**Table 1.** Electroantennogram responses of male *Opogona sacchari* moths to candidate attractants. Replicates consisted of sequential puffs in the order shown, except that the order of the aldehydes was randomized in each replicate. Air was used as a negative control, while 1-hexanol was used as a positive control. Results from ten replications that showed responses to 1-hexanol are shown. Means followed by the same letter are not significantly different (Student–Newman–Keuls test;  $P < 0.05$ )

Treatment	Electroantennogram response ( $\pm$ SE) (mV)	SNK grouping
Air only	0.33 ( $\pm 0.04$ )	A
1-6:OH	2.7 ( $\pm 0.4$ )	B
<i>Z2</i> , <i>Z</i> 13-18:Ald	3.1 ( $\pm 0.6$ )	B
<i>E2</i> , <i>Z</i> 13-18:Ald	2.5 ( $\pm 0.3$ )	B
<i>Z3</i> , <i>Z</i> 13-18:Ald	1.2 ( $\pm 0.2$ )	A
<i>E3</i> , <i>Z</i> 13-18:Ald	0.8 ( $\pm 0.2$ )	A
Air only	0.34 ( $\pm 0.04$ )	A
1-6:OH	2.6 ( $\pm 0.4$ )	B

**Table 2.** Number of *Opogona sacchari* males caught in Jackson sticky traps baited with single-component chemical lures (septa loaded with 250  $\mu$ g of chemical)<sup>a</sup>

Treatment	Males trapped per day ( $\pm$ SEM)	Tukey grouping	Range over trapping periods
Virgin female (A)	1.82 ( $\pm 0.21$ )	A	0.0–9.0
Virgin female (B)	1.75 ( $\pm 0.20$ )	A	0.0–4.5
<i>E2</i> , <i>Z</i> 13-18:Ald	1.69 ( $\pm 0.49$ )	A	0.0–5.0
<i>Z2</i> , <i>Z</i> 13-18:Ald	0.70 ( $\pm 0.18$ )	B	0.0–3.3
<i>Z3</i> , <i>Z</i> 13-18:Ald	0.36 ( $\pm 0.24$ )	B	0.0–1.8
<i>E3</i> , <i>Z</i> 13-18:Ald	0.16 ( $\pm 0.06$ )	B	0.0–0.8
<i>Z2</i> , <i>Z</i> 13-18:OAc	0.08 ( $\pm 0.05$ )	B	0.0–1.7
<i>Z3</i> , <i>Z</i> 13-18:OAc	0.01 ( $\pm 0.01$ )	B	0.0–0.5
<i>E2</i> , <i>Z</i> 13-18:OAc	0.01 ( $\pm 0.01$ )	B	0.0–0.3
<i>E3</i> , <i>Z</i> 13-18:OAc	0.01 ( $\pm 0.01$ )	B	0.0–0.3
<i>Z2</i> , <i>Z</i> 13-18:OH	0.01 ( $\pm 0.01$ )	B	0.0–0.3
<i>E2</i> , <i>Z</i> 13-18:OH	0.00 ( $\pm 0.00$ )	B	0.0–0.0
<i>Z3</i> , <i>Z</i> 13-18:OH <sup>b</sup>	0.0	–	–
<i>E3</i> , <i>Z</i> 13-18:OH <sup>b</sup>	0.0	–	–

<sup>a</sup> Attractants were assayed using pairs of traps monitored every 3–4 days over 2–5 trapping periods (one replication). Four replications were carried out over time.

<sup>b</sup> Results shown for comparison purposes only. These chemicals were tested in only one replication.

Best catch was obtained in the two experimental controls (identical treatments) using virgin females (1.75–1.82 MTD) and in traps containing *E2*, *Z*13-18:Ald (1.69 MTD). *E2*, *Z*13-18:Ald caught more than twice as many moths as did the *Z2*, *Z*13-18:Ald (0.70 MTD), the next best attractant. This difference was significant (Tukey's HSD,  $P = 0.05$ ,  $df = 80$ ) (Table 2). Other single-component chemical lures tested caught about half as many moths as *Z2*, *Z*13-18:Ald or less. As mentioned previously, *E3*, *Z*13-18:OH and *Z3*, *Z*13-18:OH were tested in only one of the four experimental replications. Over the ~2 week exposure period, no male moths were collected in any of the four traps associated with these treatments (Table 2). For all attractants, temporal variation in trap catch was extremely high. For example, the maximum number of moths captured in a trap during any of the 3–4 day trapping periods was

**Table 3.** Number of *Opogona sacchari* males caught in Jackson sticky traps baited with single-component or binary mixtures of chemical lures (septa loaded with 250 µg of chemical)<sup>a</sup>

Treatment (ratio)	Males trapped per day (± SEM)	Tukey grouping	Range over trapping periods
Virgin female (B)	0.79 (±0.45)	A	0.0–5.0
E2,Z13-18:Ald	0.78 (±0.12)	A	0.0–2.5
Virgin female (A)	0.63 (±0.18)	A	0.0–4.8
E2,Z13-18:Ald/E2,Z13-18:OH (1:1)	0.58 (±0.17)	A	0.0–3.7
E2,Z13-18:Ald/E2,Z13-18:OH (100:1)	0.49 (±0.23)	A	0.0–1.8
Z2,Z13-18:Ald/Z2,Z13-18:OH 10:1	0.47 (±0.15)	A	0.0–3.8
E2,Z13-18:Ald/E2,Z13-18:OH (10:1)	0.37 (±0.20)	A	0.0–3.3
Z2,Z13-18:Ald/Z2,Z13-18:OH (100:1)	0.25 (±0.12)	A	0.0–3.0
Z2,Z13-18:Ald/Z2,Z13-18:OH (1:1)	0.25 (±0.05)	A	0.0–1.5
Z2,Z13-18:Ald	0.22 (±0.07)	A	0.0–1.3

<sup>a</sup> Attractants were assayed using pairs of traps monitored every 3–4 days over 2–5 trapping periods (one replication). Four replications were carried out over time. The main-effects ANOVA comparing male captures in traps containing E2,Z13-18:Ald versus those containing Z2,Z13-18:Ald was significant ( $P = 0.02$ ,  $F = 6.0$ ,  $df = 1, 58$ ).

9.0, 4.5 and 5.0 MTD for the three best-performing treatments [Virgin female (B), E2,Z13-18:Ald and Virgin female (A)]. During other trapping periods, traps baited with these same attractants sometimes caught no moths at all (Table 2). Variation over trapping replications (comprising 2–5 trapping periods each) was relatively low by comparison (SEM values) (Table 2). There was evidence that the specific location of individual traps was an important variable affecting trap catch. The mean difference between trap pairs holding the same attractant was +1.5 moths per trapping period  $\pm 0.92$  (95% confidence interval).

**Field test 2 (blends of E2,Z13-18:Ald and Z2,Z13-18:Ald).** The average catch of males per day in glue traps across treatments ranged from 0.79 to 0.22 (Table 3). The number of males caught per day in traps was significantly associated with trapping row ( $P = 0.01$ ,  $F = 6.8$ ,  $df = 1, 66$ ) but not replication ( $P = 0.3$ ,  $F = 1.2$ ,  $df = 3, 66$ ) or treatment (lure type) ( $P = 0.18$ ,  $F = 1.5$ ,  $df = 9, 66$ , ANOVA results). Consistent with the first field test, the treatments that caught the most moths were the two replications of the virgin female treatment (catching 0.79 and 0.63 MTD) and the E2,Z13-18:Ald when used alone (0.78 MTD), which caught more than 3 times as many males as Z2,Z13-18:Ald used alone (0.22 MTD) (Table 3). A multiple comparison test indicated no significant differences in trap catch among any of the treatments (Tukey's HSD,  $\alpha = 0.05$ ) (Table 3). However, the main-effects ANOVA comparing male captures in traps containing E2,Z13-18:Ald versus those containing Z2,Z13-18:Ald was significant ( $P = 0.02$ ,  $F = 6.0$ ,  $df = 1, 58$ ). The lures containing E2,Z13-18:Ald caught an average of 0.55 MTD, while those containing Z2,Z13-18:Ald caught 0.30 MTD.

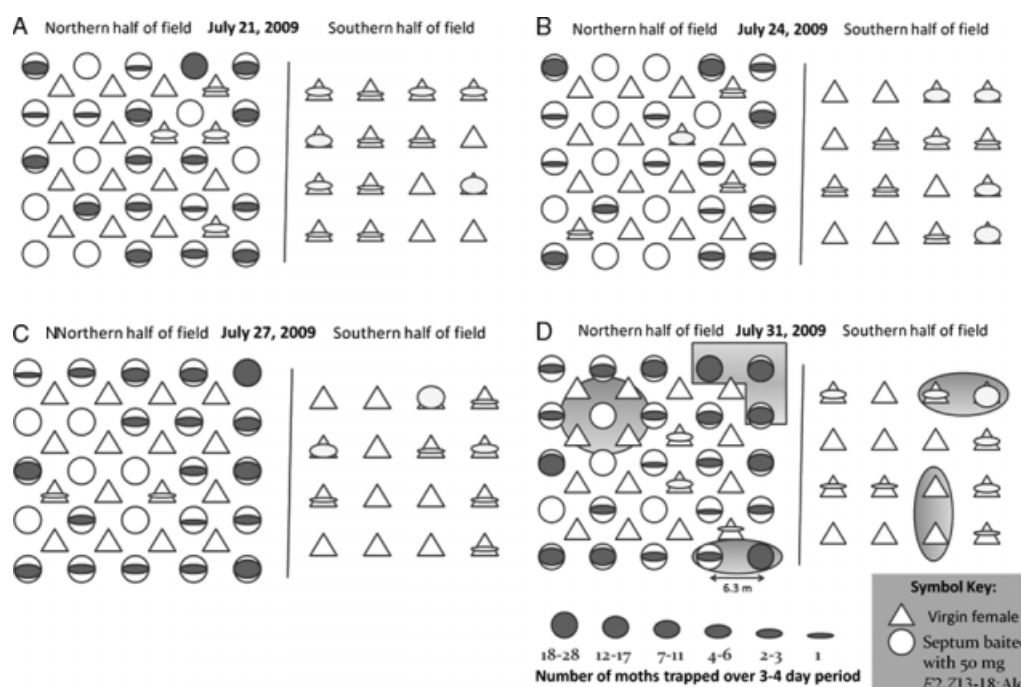
**Field test 3 (mass trapping trial using E2,Z13-18:Ald).** The average number of male moths captured in Jackson traps holding virgin females in the mass-trapped area versus the control area was

0.09 versus 0.46 MTD, respectively, over the 2 week collection period. This compared with 1.04 MTD in the traps used for mass trapping that held the artificial lure. Expressed as a percentage of catch in traps holding the artificial lure, trap catch in the 16 Jackson traps baited with virgin females in the northern half of the field averaged 14, 22, 4 and 5% for collection dates of 21, 24, 27 and 31 July respectively (Fig. 1). The  $t$ -tests indicated that traps in the northern area of the field holding virgin females caught significantly fewer moths than traps baited with the artificial lure on each of the four sample dates ( $P$  values all  $<0.05$ ,  $df = 39$ ). Based on an examination of the spatial distribution of trap catches, it was possible to identify clusters of trap locations that were consistently associated with high or low catches over the four collection periods. These 'hot' and 'cold' spots are indicated via shading in Fig. 1(D).

## 4 DISCUSSION

Based on the GC-MS, EAG and field data collected, the authors tentatively identify E2,Z13-18:Ald as an attractant produced by *O. sacchari* female moths. While the retention time of the 2/3,Z13-18:Ald matched that of E2,Z13-18:Ald, precise identification becomes difficult because many of these aldehydes are unstable and isomerize during GC analysis (even with cool on-column injection).<sup>22</sup> Islam *et al.*<sup>22</sup> developed an HPLC technique that can detect the conjugated 2,13-dienals at nanogram quantities. However, 3,13-dienals lack a strong chromatophore and must be derivatized with a hydrazine reagent before analysis. As the aim of the present work was to identify an attractant that could be used in the field, the authors chose to use electrophysiology and field testing to find the most active attractant(s) among the candidate 2/3,(Z)13-octadecadienyl compounds.

While GC-MS analysis was unable to assign stereochemistry to the isolated 2/3,Z18-dienal, the field data showing that E2,Z13-18:Ald is the most active isomer strongly suggest that this is the active compound released by calling female moths. Field catches with Z2,Z13-18:Ald are more difficult to interpret as the compound may be attractive to male *O. sacchari*, males may be attracted to the 4% E2,Z13-18:Ald present in the lure and/or Z2,Z13-18:Ald may be isomerized to E2,Z13-18:Ald under field conditions. Additionally, it is possible that the actual pheromone released by female moths is a mixture of aldehyde isomers. EAG data did not show a difference between responses to E2,Z13-18:Ald and Z2,Z13-18:Ald, indicating that both compounds are perceived by male moths. From a practical standpoint, E2,Z13-18:Ald is the more stable isomer, is much easier to synthesize (PCC as opposed to Dess–Martin oxidation) and is therefore the best choice as a sex attractant for *O. sacchari* population monitoring and control applications. The first field test demonstrated that E2,Z13-18:Ald used alone was attractive at low concentrations and caught significantly more *O. sacchari* in survey traps than any of the other chemicals tested singly. In the second field test, which included blends, E2,Z13-18:Ald used alone again caught the most moths, although none of the lure types differed significantly from the others. The lack of statistical separation might be partly due to generally lower trap catches in this test period relative to the first, a situation expected to increase within-treatment variability. Regardless, the results of field test 1 were confirmed by the main-effects test for field test 2, which demonstrated that lures containing E2,Z13-18:Ald caught significantly more male moths than those containing Z2,Z13-18:Ald.



**Figure 1.** Graphical and spatial representation for the number of *Opogona sacchari* collected in Jackson traps holding either a virgin female moth or a septum dosed with 50 mg of *E2,Z13-18:Ald*. Traps of the same type were spaced 6.3 m apart. The key to the symbols used is shown in Fig. 1D. The shaded areas enclosing groups of traps indicate trap locations where the trap catch was either consistently high or consistently low over the 2 week sampling period.

Based on the cost for the synthesis of the *E2,Z13-18:Ald* lure, the cost of the chemical in each septum dosed with 250 µg was \$US 0.10. These lures provided effective attraction in the field for more than 2 weeks under east Hawaii conditions. Recent tests using septa supplied by a cooperator (data not shown) suggest that this 2 week period can be extended to at least 8 weeks when appropriate chemical stabilizers are added to the *E2,Z13-18:Ald*. Using the synthetic pheromone to attract males is a simpler and lower-cost option compared with using virgin females. Producing virgin females is time intensive and expensive. Further, these insects are difficult to handle because they escape easily when being transferred into cages and are easily killed if exposed to direct sunlight within a closed container. The results of the mass trapping trial indicate the potential of using this technique for population control of banana moth. Although it proved impossible completely to shut down trap catch in traps holding virgin females, the traps holding the artificial lure, interspersed with those holding virgin females, caught more than 10 times as many moths. The authors plan to repeat this trial using a PVC or polyethylene dispenser, which may release the attractant more quickly. The half-life of various moth pheromones loaded onto natural rubber septa (like the ones used in the present trial) was studied by Butler and McDonough.<sup>26</sup> Half-life increased dramatically as the number of carbon atoms in the molecule increased. Alcohols having 12 carbon atoms had half-lives ranging from 14 to 45 days, while those with 14 carbons had half-lives ranging from 117 to 202 days. Alcohols having 16–18 carbons had half-lives of 399–1117 days. Although Butler and McDonough did not test *E2,Z13-18:Ald*, it is reasonable to surmise that the bulk of this 18-carbon material was not released from septa during the present 2 week study period. Prior to the mass trapping experiment described in this manuscript, the authors also carried out mating disruption and mass trapping trials in the same papaya field using septa loaded

with 10 mg of *E2,Z13-18:Ald*. While the mating disruption trial was unsuccessful, mass trapping was a partial success, although not as successful as the trial reported in this manuscript. The fact that the 50 mg dose rate provided better mass trapping results than the 10 mg dose rate using an identical placement of traps suggests that the failure to shut down attraction to virgin females could be remedied by increasing the release rate of the attractant.

In the mass trapping trial and in other field studies described in this manuscript, it was observed that certain traps consistently caught relatively larger numbers of *O. sacchari* males (5–10-fold more) than adjacent traps that were less than 7 m away. Such patterns sometimes persisted over a period of weeks, after which other trap locations began to catch relatively large numbers. Based on these data, it was hypothesized that most of the males that were being caught in traps emerged as adults very close to the traps in which they were caught. In the abandoned papaya field used in earlier studies, counts of *O. sacchari* larvae in rotting papaya trunks adjacent to high- and low-trap-catch areas were made during timed searches. The results were consistent with the given hypothesis, i.e. larval counts were higher adjacent to traps that regularly caught greater numbers of moths (data not shown). This suggests that males have a low dispersal rate or are efficiently caught soon after emergence as adults or have a relatively low sensitivity and short active-space response range to this lure. If mating disruption or mass trapping can be achieved using *E2,Z13-18:Ald*, this technology is expected to be useful on the scale of individual farms, as reinvasion following successful control might be relatively slow.

## ACKNOWLEDGEMENTS

The authors would like to thank Janice Nagata, John Ross and Esther Schneider for their assistance in conducting field



experiments, electrophysiological experiments and moth rearing, Randy Akao (WH Shipman Limited) and Patrick McGrath (Hawaii Nurseries, Inc.) for permission to carry out tests in papaya and *Dracaena* fields, Scentry Biologicals for samples of (Z,Z)-3,13-octadecadienol, the Shenandoah Valley NMR Consortium housed at James Madison University for use of  $^1\text{H}$  NMR and Douglas Light and Aijun Zhang for comments on an earlier manuscript. This project was funded by a grant from the Hawaii Department of Agriculture (Agreement No. 58-5320-7-423).

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